Antimicrobial Activity of 8-Quinolinol, its Salts with Salicylic Acid and 3-Hydroxy-2-Naphthoic Acid, and the Respective Copper (II) Chelates in Liquid Culture

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ABSTRACT

GERSHON, HERMAN (Pfister Chemical Works, Inc., Ridgefield, N.J.) AND RAULO PARMEGIANI. Antimicrobial activity of 8-quinolinol, its salts with salicylic acid and 3-hydroxy-2-naphthoic acid, and the respective copper (II) chelates in liquid culture. Appl. Microbiol. **11**:62–65. 1963.—A study was made of the antimicrobial activities of 8-quinolinol and its salts with salicylic acid and 3-hydroxy-2-naphthoic acid in liquid culture. Comparisons of results were made with those obtained by the disc-plate method. The mechanism of action of copper chelates was discussed.

In earlier reports (Gershon and Parmegiani, 1962; Gershon, Parmegiani, and Nickerson, 1962), it was indicated that a considerable number of salts of the 8-quinolinols with aryl hydroxy acids showed equal or superior antimicrobial activity to the parent quinolinol by the disc-plate method in a screening system employing strains of five bacteria and five fungi. The corresponding copper(II)-chelated compounds, when examined by the same technique, allowed the following generalizations to be drawn. (i) Chelation of the aryl hydroxycarboxylic acids with copper had very little effect on antimicrobial activity. (ii) Chelation of 8-quinolinol with copper reduced antimicrobial activity. (iii) Chelation of 8-quinolinolium aryl hydroxycarboxylates with copper had little effect on antibacterial activity, but greatly enhanced antifungal activity.

These results warranted re-examination of the materials in liquid culture for comparison with the disc-plate method, to obtain quantitative antimicrobial data on the compounds, and to add, if possible, to the understanding of the mechanisms of action of the chelates. This is the subject of the present report.

MATERIALS AND METHODS

The organisms employed in our screening system included five fungi (*Aspergillus niger*, *Trichophyton mentagrophytes*, *A. oryzae*, *Myrothecium verrucaria*, and *Trichoderma viride*) and five bacteria (*Escherichia coli* *Pseudomonas aeruginosa*, *Leuconostoc mesenteroides* P-60 *Staphylococcus aureus*, and *Streptococcus faecalis*).

The compounds that were selected for study were from the tables of materials screened by Gershon and Parmegiani (1962) and Gershon et al. (1962) and are listed in Table 1.

Media in liquid culture method. For the antifungal testing, the following media were employed: Sabouraud's liquid culture medium (Difco) enriched with 0.05% yeast extract (Difco) for *A. niger*, *A. oryzae*, *M. verrucaria*, and *T. viride*, and for *T. mentagrophytes*, the above medium also enriched with 10% beef serum (Difco). For the bacterial studies, all organisms were cultured on Trypticase Soy Broth (BBL). The medium for *S. aureus* was not enriched with beef serum because growth was measured turbidimetrically, and the turbidity due to the serum was not desired.

Method of testing in liquid culture method. The antifungal tests were conducted as follows. To 98 ml of the sterile Sabouraud's liquid culture medium was added, aseptically, 1 ml of dimethyl sulfoxide containing concentrations of compound to yield graded levels up to 100 ppm when diluted to 100 ml. A 1-ml amount of inoculum was added which contained 6×10^6 spores in 0.85% saline. The flasks were shaken at 250 oscillations/min on a shaker with a 1-in. eccentric rotary motion at 28 C. After 6 days, the presence or absence of growth was recorded. All flasks showing no apparent growth were further tested for fungicidal and fungistatic effects on the spore inoculum. A 1-ml sample of medium which showed no growth was added to 15 ml of sterile Sabouraud's liquid culture medium and incubated at 28 C for 2 weeks. If the next lower level of compound was also inhibitory, then it was necessary to dilute the test sample of medium below the concentration of compound at the next lower level.

The antibacterial tests were conducted as follows. To 5.9 ml of sterile Trypticase Soy Broth was added, aseptically, 0.1 ml of dimethyl sulfoxide containing graded levels of compound up to 100 ppm at the final dilution in Klett tubes. One drop of inoculum was added to each tube, followed by incubation at 37 C for 3 days. Growth was indicated by turbidity as read in a Klett-Summerson

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colorimeter, using a no. 66 filter. The inoculum was prepared from 18-hr cultures of the organisms in Trypticase Soy Broth which had been sedimented by centrifugation, resuspended in 0.85% saline, and standardized in the colorimeter to a turbidity reading of 180 Klett units. Bacteriostatic and bactericidal determinations were carried out by adding two loopfuls of the apparently inhibited culture to 15 ml of sterile Trypticase Soy Broth and incubating at 37 C for 3 days. When growth was observed, the compound under study was considered bacteriostatic but when no growth appeared on subculture, the test compound was considered bactericidal.

The test intervals of compound for both the fungal and bacterial procedures were arranged in such a manner that the final inhibitory result was determined to the nearest 1 ppm. The fungicidal and bactericidal levels were determined to the nearest 5 ppm.

Results

Tables 1 and 2 summarize the antifungal and antibacterial results, respectively. The salts of 8-quinolinol are not as active against these organisms as 8-quinolinol alone in liquid culture. Also, the aryl hydroxy acids appear to be inactive under the conditions of these tests. It seems that the activity of the salt is in proportion to the 8-quinolinol content alone, for both the fungi and the bacteria. In the case of the copper-chelated compounds, the unexpected observation was made that all the materials were nearly equal in activity against both the fungi and the bacteria. Finally, it was also determined that the copper salts of salicylic acid and 3-hydroxy-2-naphthoic acid were inactive against the fungi under these test conditions. It should also be noted that since the molecular weights of 8-quinolinol and the aryl hydroxy acids are relatively close, the use of the units ppm would not cause a significant error when the activity of the chelate compounds is discussed on a molecular basis.

Discussion

In our earlier work (Gershon and Parmegiani, 1962), we reported that 8-quinolinol and its salts with the unsubstituted aryl hydroxycarboxylic acids were equal

TABLE 1. *Antifungal activity (ppm) of 8-quinolinol, its salts with aryl hydroxy acids, and the respective copper (II) chelates in Sabouraud's liquid culture medium at 28 C after 6 days in shake flasks*

No.	Compound	*A. niger*		*A. oryzae*		*T. viraide*		*M. verrucaria*		*T. mentagrophytes**	
		S†	C	S	C	S	C	S	C	S	C
1	8-Quinolinol	10	>100	17	>100	2	8	6	6	4	4
2	8-Quinolinolium salicylate	20	>100	44	>100	3	50	10	25	5	25
3	8-Quinolinolium 3′-hydroxy-2′-naphthoate	25	>100	45	>100	5	50	14	14	5	6
4	8-Quinolinolium 1′-hydroxy-2′-naphthoate	25	>100	50	>100	5	50	15	25	5	50
5	8-Quinolinol, Cu(II) chelate	2	50	4	50	2	50	3	3	2	2
6	8-Quinolinolium salicylate, Cu(II) chelate	3	50	4	>50	3	>50	6	6	2	2
7	8-Quinolinolium 3′-hydroxy-2′-naphthoate, Cu(II) chelate	3	50	4	50	3	50	4	4	2	2
8	8-Quinolinolium 1′-hydroxy-2′-naphthoate, Cu(II) chelate	3	50	4	50	2	50	4	4	2	2
9	Salicylic acid	>100		>100		>100		>100		>100	
10	3-Hydroxy-2-naphthoic acid	>100		>100		>100		>100		>100	
11	Copper(II) salicylate	>100		>100		>100		>100		>100	
12	Copper(II) 3-hydroxy-2-naphthoate	>100		>100		>100		>100		>100	

* Medium enriched with 10% beef serum.
† S = fungistatic; C = fungicidal.

TABLE 2. *Antibacterial activity (ppm) of 8-quinolinol, its salts with salicylic acid and 3-hydroxy-2-naphthoic acid, and the respective copper (II) chelates at 37 C after 3 days in Trypticase Soy Broth*

No.	Compound	*E. coli*		*S. aureus*		*S. faecalis*		*P. aeruginosa*		*L. mesenteroides*, P-60	
		S*	C	S	C	S	C	S	C	S	C
1	8-Quinolinol	59	100	4	4	>100		>100		100	>100
2	8-Quinolinolium salicylate	>100		8	10	>100		>100		>100	
3	8-Quinolinolium 3′-hydroxy-2′-naphthoate	>100		8	8	>100		>100		>100	
5	8-Quinolinol, Cu(II) chelate	>100		4	4	11	11	>100		11	15
6	8-Quinolinolium salicylate, Cu(II) chelate	>100		5	5	12	12	>100		13	14
7	8-Quinolinolium 3′-hydroxy-2′-naphthoate, Cu(II) chelate	>100		5	5	8	10	>100		12	15

* S = bacteriostatic; C = bactericidal.

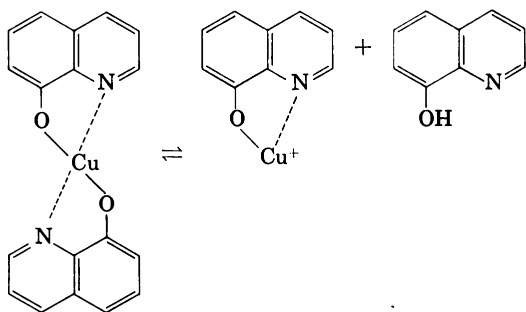
in activity by the disc-plate method. This conclusion was not borne out by the liquid-culture studies. This difference may be explained, in part, by the fact that the sensitivity of the liquid-culture methods, as herein described, is much greater than that of the disc-plate method. Consequently, the critical levels of both types of compounds fell within the ranges selected for the preparation of discs, and no apparent differences were found between 8-quinolinol and its salts. Furthermore, the physical properties, such as greater water solubility and lower volatility of the salts, are advantageous in the disc-plate system.

As previously mentioned, the copper chelates of 8-quinolinol and its salts with aryl hydroxy acids possess equal antimicrobial activity in the liquid-culture systems employed. This is not in agreement with the results obtained by the disc-plate method (Gershon et al., 1962). The differences in activity of these compounds in both test systems might conceivably be explained by the greater water solubility of compounds 6 to 8 as compared with compound 5 (Tables 1 and 2), although such data are not presently available.

In attempting to understand the mechanism of action of the copper chelates, a number of points are now considered important. The activity of 8-quinolinol seems to be due to metal chelate formation, and the toxic effect takes place within the cell. Chelation is not sufficient for toxicity; it must be accompanied by lipid solubility (Albert, Gibson, and Rubbo, 1953; Albert et al., 1954; Block, 1955).

It can be seen that the antifungal effect of the copper chelates of salicylic acid and 3-hydroxy-2-naphthoic acid was no greater than that of the parent acids (Table 1). This was to be expected owing to the water solubility of the copper chelates. On the other hand, the mixed chelates showed comparable activity to copper(II) 8-quinolinolate, and these are lipid-soluble.

Albert et al. (1953) proposed that the 1:2 chelate of copper(II) 8-quinolinate penetrated the cell and dissociated to a 1:1 half chelate and free 8-quinolinol.



The half chelate became the toxic entity by combining with, and blocking, metal-binding sites on enzymes. Block (1955) proposed that natural chelators within the cell were poisoned by removing copper from copper (II) 8-quinolinolate, thereby freeing 8-quinolinol which could then bind the metallic prosthetic groups from enzymes. Zentmyer, Rich, and Horsfall (1960) proposed a mechanism of detoxication of the 1:2 chelate by natural metabolites. The 1:2 chelate dissociates to the 1:1 chelate, thereby entering the aqueous phase of the cell. Histidine and cysteine, which form more stable complexes with copper than the 1:1 chelate of copper, and 8-quinolinol, remove the copper and form lipid-soluble chelates. Esposito and Fletcher (1961) proposed that the activity of copper(II) 8-quinolinolate was due to the 1:1 complex which could bind with an enzyme site that was involved in the biosynthesis of pteridines. This was based on the reversal of inhibition by several pteridines and precursors.

Most of these mechanisms of action have in common the 1:1 complex of copper and 8-quinolinol.

In attempting to explain the activity of the mixed chelates, it should be pointed out that all of the compounds possess approximately equal percentages of copper, the composition which could yield the 1:1 complex, but only approximately half of the 8-quinolinol present in copper(II) 8-quinolinolate.

If the hypothesis of Block (1955), regarding the freeing of 8-quinolinol from the complex by natural chelators, were true, then the mixed chelates should have been only about one-half as active as 8-quinolinol copper(II) chelate, since the avidity of the aryl hydroxy acids for heavy metals is of a much lower order of magnitude than that of 8-quinolinol. This was not found to be the case.

The results obtained with the mixed chelates can be interpreted by the half-chelate hypothesis of Albert et al. (1953); however, no distinction can be made with these data between the detoxication mechanisms proposed by Zentmyer et al. (1960) or Esposito and Fletcher (1961).

The present work can be summarized by the following conclusions. (i) The activity of the 8-quinolinolium salts is due primarily to the 8-quinolinol content. (ii) The antimicrobial activity of copper(II) 8-quinolinolate is not due to the release of 8-quinolinol within the cell, which in turn binds heavy-metal prosthetic groups from enzymes. (iii) The toxicity of copper(II) 8-quinolinolate is not due to the entire molecule. A portion such as the 1:1 chelate, as proposed by Albert et al. (1953), is all that is required for toxicity toward microorganisms. (iv) Based on the results with eight organisms, it appears that the mechanism of action of these copper chelates is universal.

Additional work with the mono- and dihalogenated 8-quinolinols and their respective copper chelates is in progress.

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